Development of Strategies and Methods for Monitoring for Algal Blooms and Occurrence of Toxic Cyanobacteria Using Next Generation qPCR and Phylochip Microarrays

Laura Webb, EPA Region 7

EPA Region 7, Laboratory Services and Applied Sciences Division, Field Services Branch
Kansas City, KS

March 27, 2019

Project Team

- Laura Webb, Regina Klepikow, Steven Baker, Amy Shields, EPA Region 7
- Eric Villegas, Jingrang Lu – ORD Exposure Methods and Measurements, National Exposure Research Laboratory, Microbial Exposure Branch
- Heath Mash – ORD National Risk Management Research Laboratory, Water Systems Division, Drinking Water Systems Branch

Regional Applied research effort (RARE)

- Collaborative effort between and ORD office or lab and a regional office or lab
- Any high priority research need that the region has and that ORD has the expertise to address
- Each region nominates projects and selects, based on merit and funding, which ones move forward
- 2 year project in 5 regional lakes

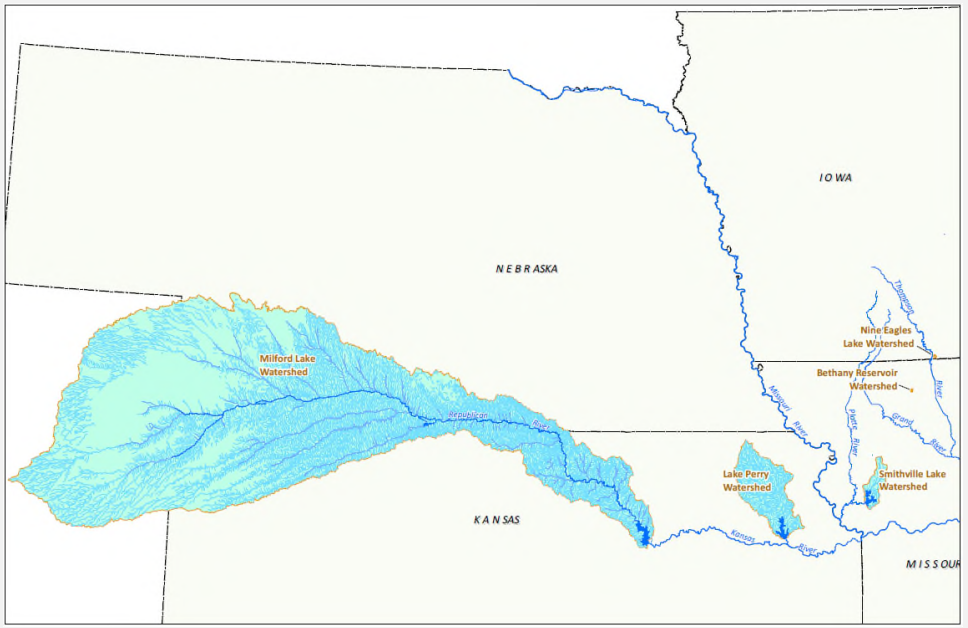
Goals

- Purpose is to develop a good screening tool to determine if and what genus and or species of cyanobacteria are present and to determine if they are capable of producing toxins
- qPCR (R7) and the qPCR/RT-qPCR (ORD)
  - Using a commercial kit (CyanoDTec) will yield Total Cyanobacteria, Toxin Producing Genes (Microcystin/Nodularin, Saxitoxin, Cylindrospermopsin)
    - Pro: It's a commercially available kit that has gone through NIST equivalence testing and certification *product of Australia
    - Con: Not sure if the gene targets used are the same as those that reside here in the US and can cost $60/sample to analyze
  - ORD using RT-qPCR
    - Pro: They have many studied methods, gene targets and research to verify the validity of the CyanoDTec
    - Con: They don't have a standardized protocol/method
  - Correlate molecular and biological results with chemical results

Phylochip and High Throughput Sequencing

- The high throughput sequencing will be used to build a whole genome map of the microbial community
- Alongside the Phylochip, we will look for any connections in the community composition and bloom formation/toxicity
- With PhyloChip we are also trying to evaluate the types of cyanobacteria from Phylum down to species.
  - Phylochip has over 800 cyanobacteria listed
- With the Phylochip we are also trying to develop the Microbial source tracking capabilities
- Work is still being done on this and not all data has been received and or interpreted

Sites



- Five lakes chosen within easy driving distance to STC
- Two lakes chosen to represent “reference” conditions, or less impacted area (Bethany, MO and 9 Eagles State Park, IA)
- Three lakes chosen to represent known HAB locations (Smithville Lake, MO, Milford and Lake Perry, KS)

EPA United States Environmental Protection Agency

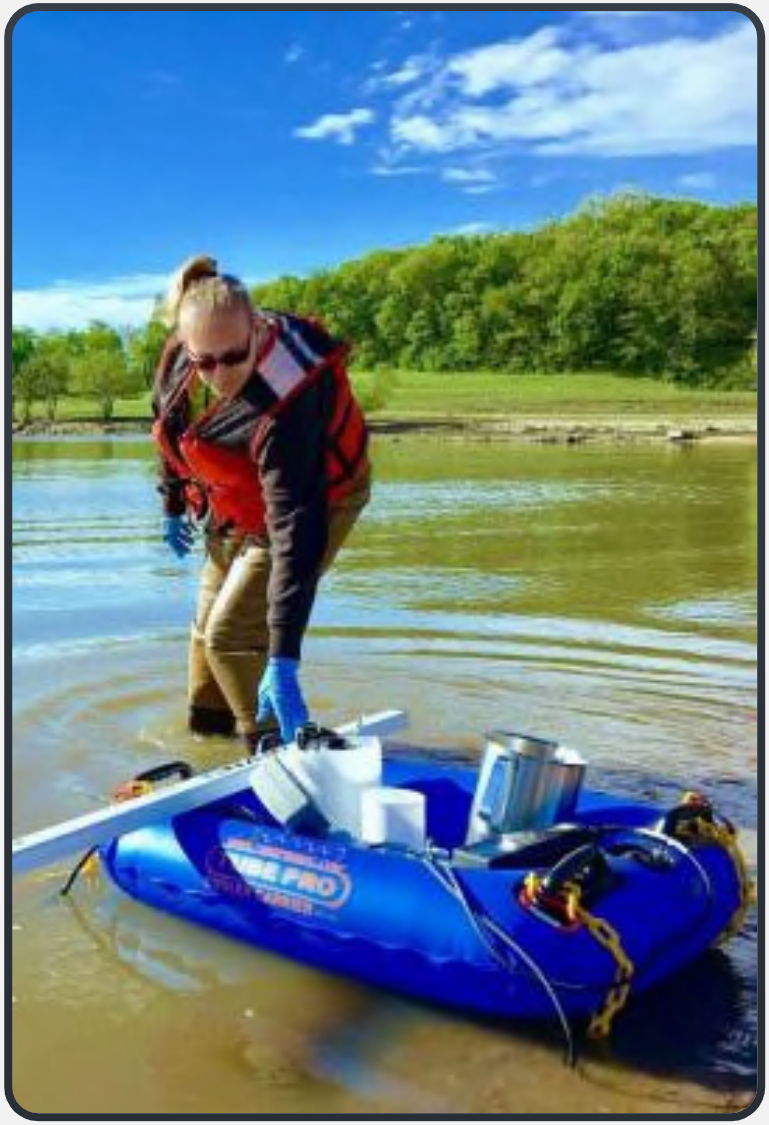
Watershed Characteristics

Land Cover Type	Open Water	Forest	Shrub/Scrub	Herbaceous	Wetlands	Dev, Open Space	Dev, Low Intensity	Dev, Med Intensity	Dev, High Intensity	Pasture/ Hay	Crop
Nine Eagles Lake	6.79	78.37	0.08	2.08	0.43	4.54	0.56	0.00	0.00	5.92	1.24
Bethany Reservoir	11.11	20.88		2.07	1.92	5.12	1.89	0.00	0.00	50.79	6.22
Smithville Lake	6.12	9.70	0.33	0.82	1.97	4.98	1.52	0.19	0.04	38.64	35.57
Lake Perry	2.57	12.35	0.21	8.79	1.00	3.99	0.84	0.13	0.02	45.60	24.49
Milford	0.41	0.67	0.04	42.38	0.71	3.11	0.31	0.05	0.01	0.65	51.50

EPA United States Environmental Protection Agency

Sampling Plan

- Sampled each lake weekly on Tuesday for 26 weeks
- Surface water samples at 3 locations at each beach
- Composited grab samples for chemical
- Sterile grab samples for molecular, composited post-sampling
- Phytoplankton net for taxonomy
- *In situ* using two YSI sondes

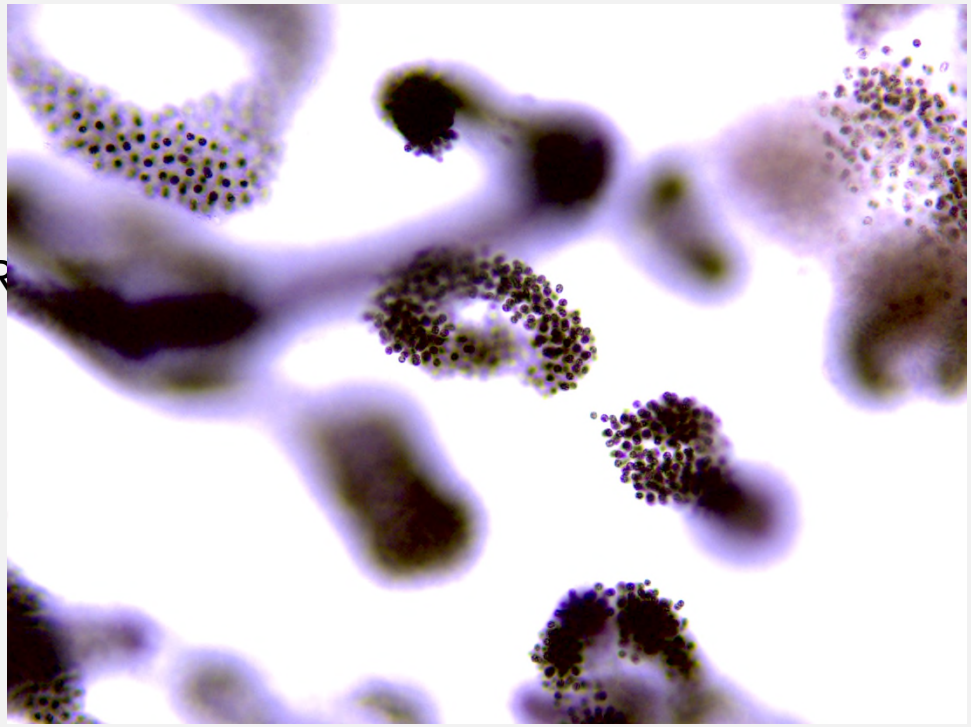


Analytes

- **Chemical**: Metals, PAH, pesticides, herbicides, pharmaceuticals, nutrients, anions, personal care products, hormones, endocrine disrupters, chlorophyll a
- **Cyanotoxins**: ELISA (MC and CYL), LCMSMS MC congeners (ORD)
- **Biological**: cyanobacteria, e coli
- **Molecular**: cyanobacteria DNA, community DNA, p-PCR
- ***In situ**:* DO, pH, turbidity, conductivity, temperature, chlorophyll a, phycocyanin

Molecular samples

- 2017
  - E.coli every 5th week
  - Phylochip every 5th week
  - ORD provided taxonomic sequencing, qPCR, and rt-qPCR
  - R7 qPCR
- 2018
  - Weekly sampling for all molecular parameters
  - R7 qPCR and E.coli
  - ORD qPCR and rt-qPCR
  - Phylochip targeted sampling



Taxonomic Identification in Bethany

- **Aphanizomenon**
- **Dolichospermum (Anabaena)**
- **Woronichina**

Cyanobacteria & Associated Toxins

Strains produce different toxins at different amounts

Toxins can have multiple variants

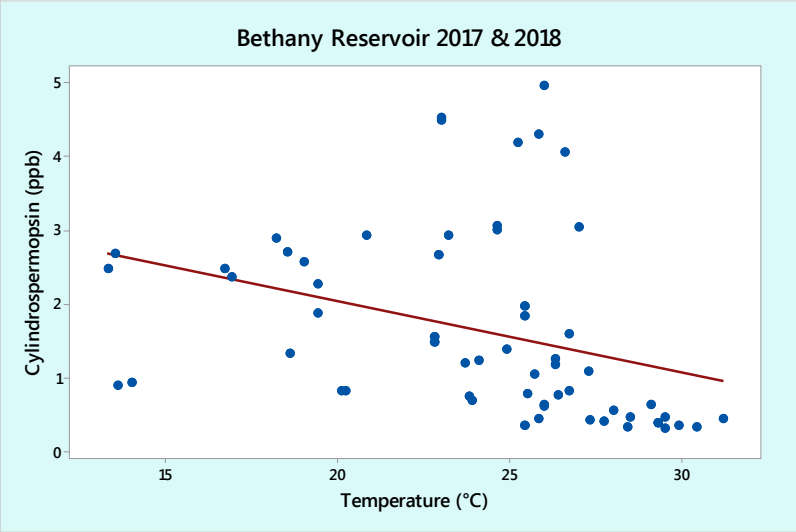
Over 80 known microcystin variants

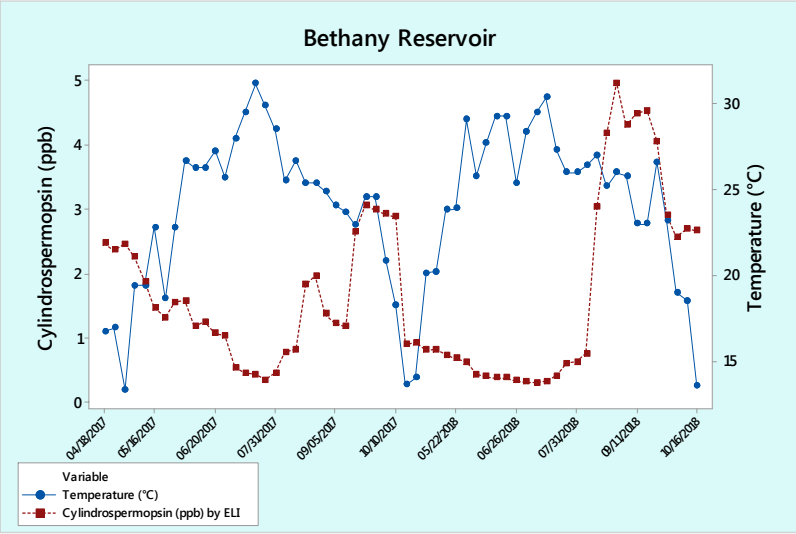
Toxin Group	Primary Target organ in mammals	Cyanobacterial genera
Microcystins	Liver	*Microcystis, Anabaena, Planktothrix (Oscillatoria), Nostoc, Hapalosiphon, Anabaenopsis*
Nodularian	Liver	*Nodularia*
Anatoxin-a	Nerve Synapse	*Anabaena, Planktothrix (Oscillatoria), Aphanizomenon*
Aplysiatoxins	Skin	*Lyngbya, Schizothrix, Planktothrix (Oscillatoria)*
Cylindrospermopsins	Liver	*Cylindrospermopsis, Aphanizomenon*
Lyngbyatoxin-a	Skin, G.I. Tract	*Lyngbya*
Saxitoxins	Nerve Axons	*Anabaena, Aphanizomenon, Lyngbya, Cylindrospermopsis*
Lipopolysaccharide	Potential irritant; affects any exposed tissue	ALL

EPA United States Environmental Protection Agency

Bethany Temperature and Toxin Relationship

- Cylindrospermopsin found only in Bethany, and always at some level in Bethany
- Found 4 years in a row here
- Highest concentration of toxin found in cooler months
- Never above the EPA Draft water quality criteria/swimming advisory of 8 ug/L
- EPA Drinking Water Health Advisory (10-day)

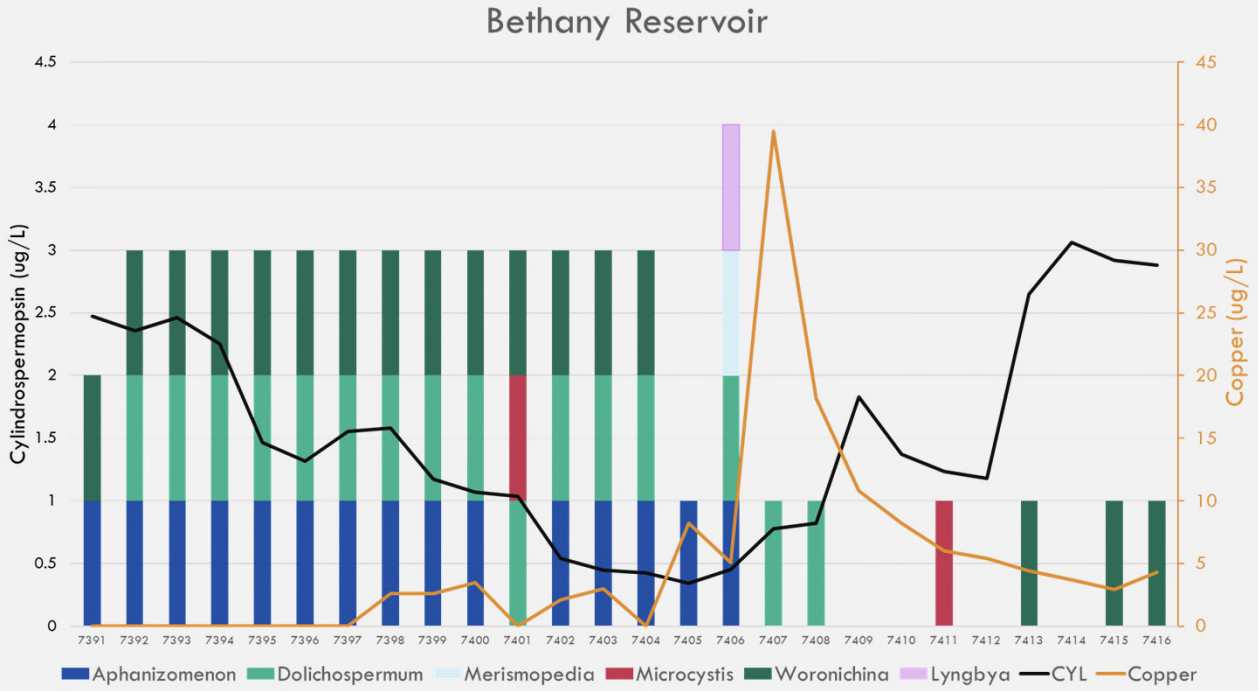




	Bottle-fed infants and pre-school children	School-age children and adults
Cylindrospermopsin	0.7 µg/L	3 µg/L

Bethany and Copper

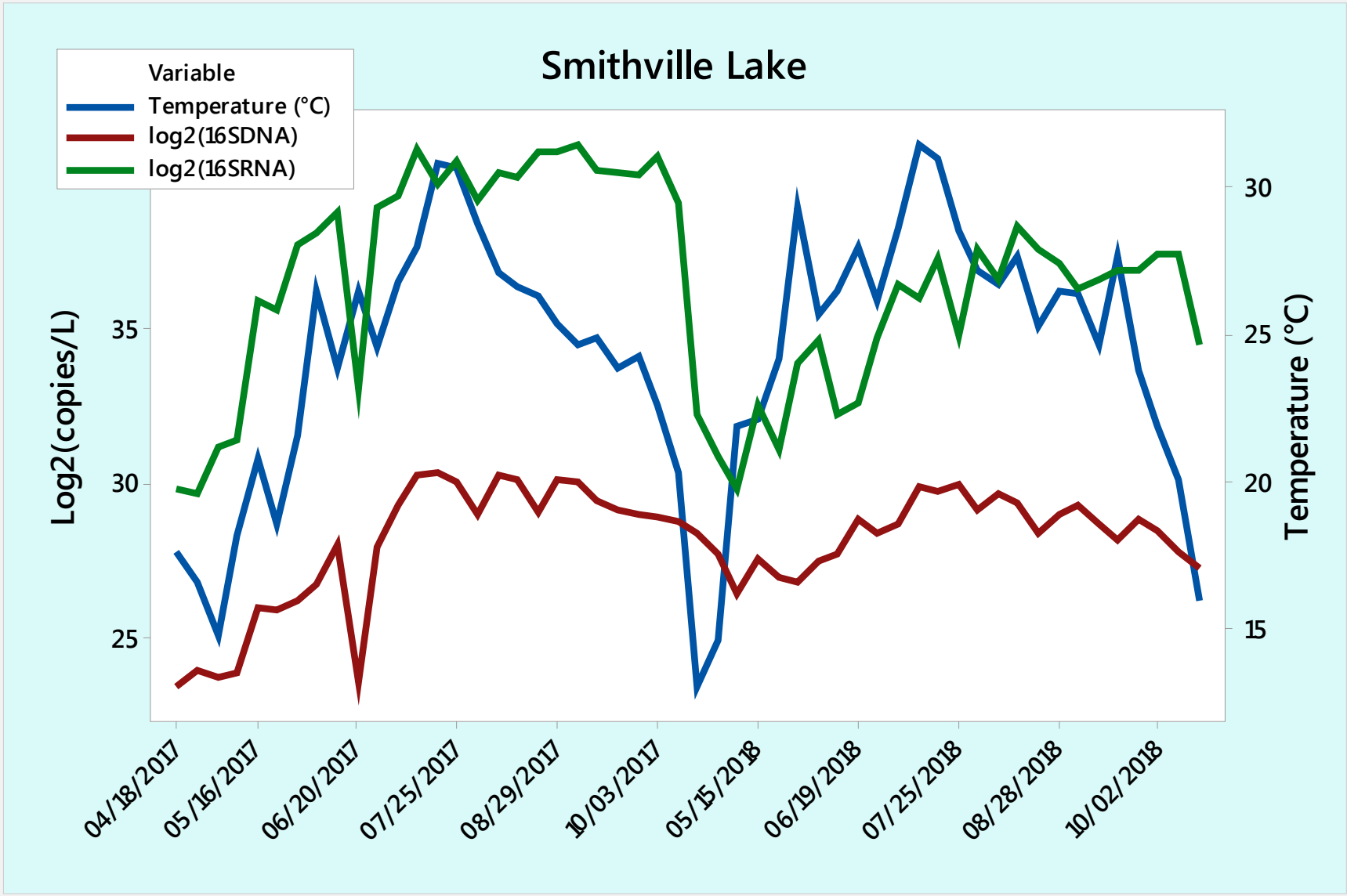
- Bethany has a significantly different concentration of copper
- Time-line of Bethany shows a drastic spike in copper – perhaps treatment with copper based algaecide
- Correlation of copper with lower chlorophyll a concentrations – killing off the algae
- Potential for lysing of cyanobacteria cells and releasing the toxin



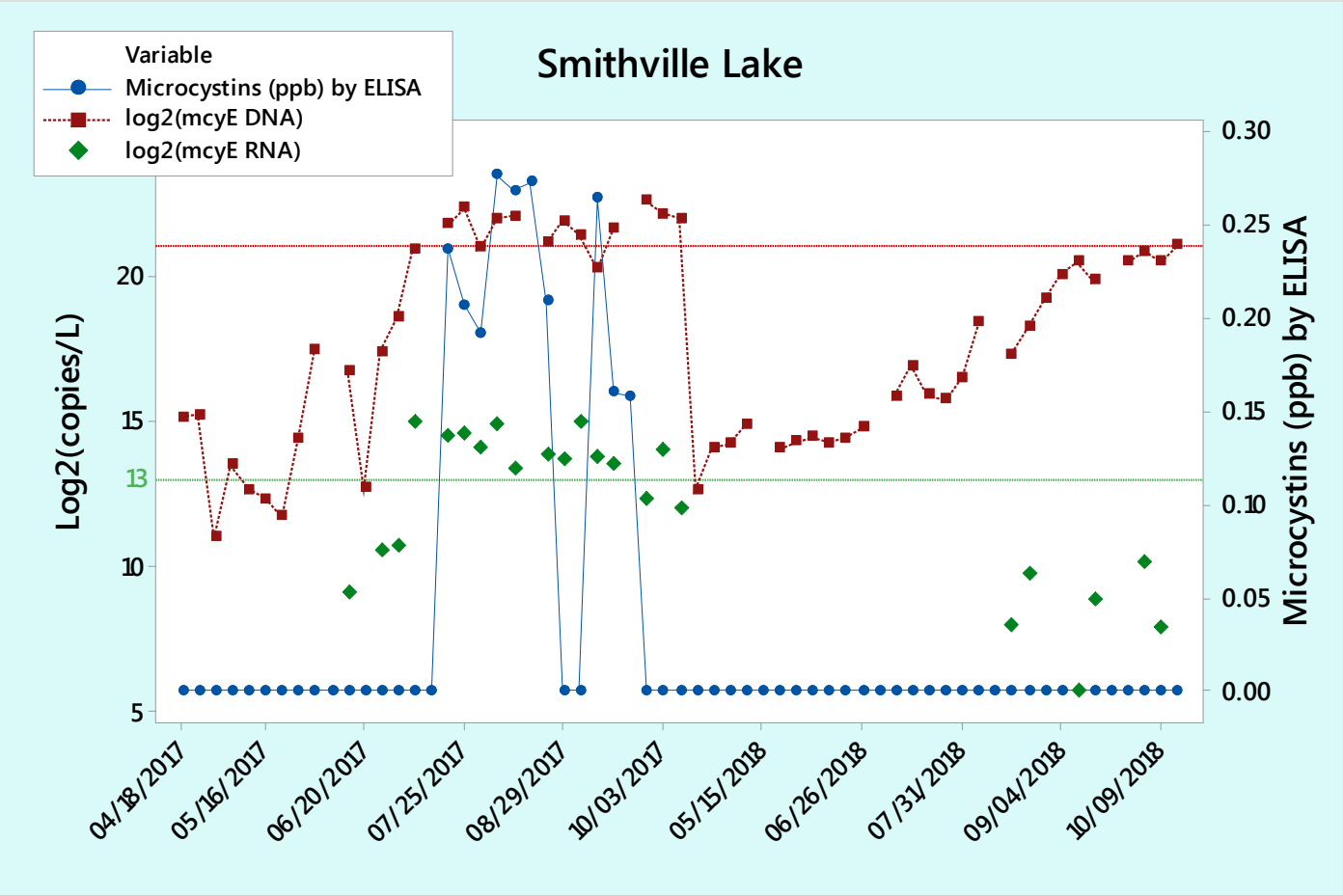
Phylochip Ave Abund before almost 9000, after was 6500. Had 107 hits before, 57 after

EPA United States Environmental Protection Agency

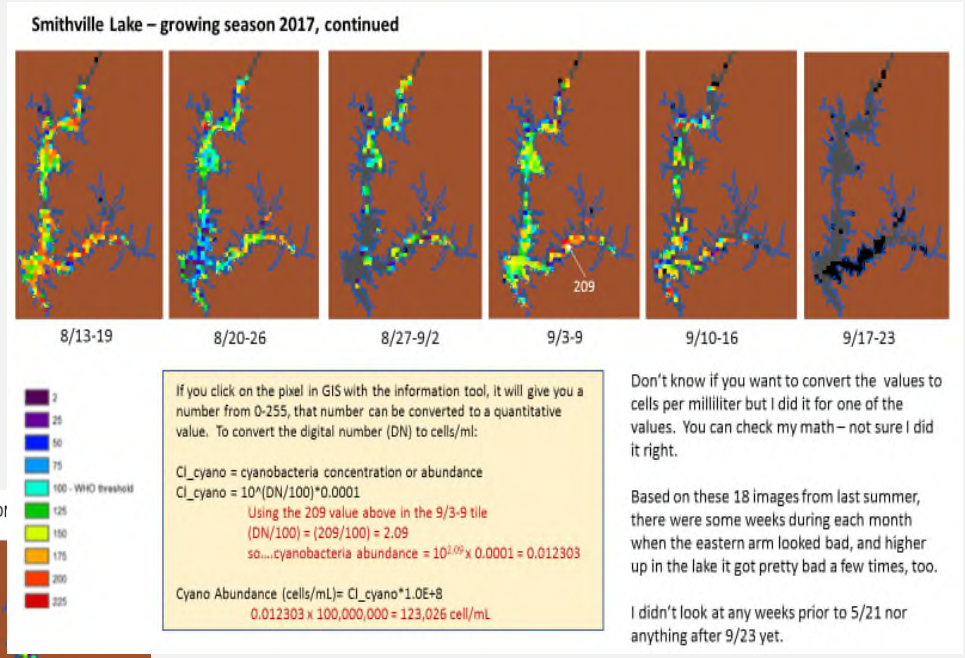
DNA Sequencing

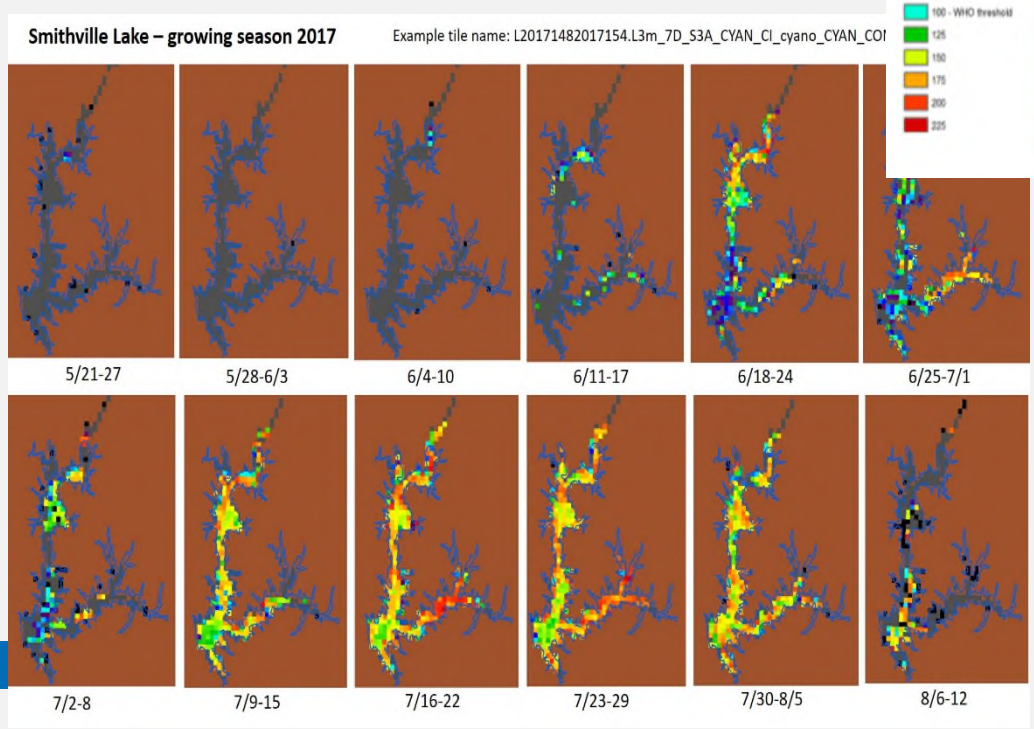

Smithville Lake
Variable
Temperature (°C)
log2(16SDNA)
log2(16SRNA)
Log2(copies/L)
35
30
25
Temperature (°C)
30
25
20
15
04/18/2017
05/16/2017
06/20/2017
07/25/2017
08/29/2017
10/03/2017
05/15/2018
06/19/2018
07/25/2018
08/28/2018
10/02/2018

Relationship between Microcystin and DNA, RNA signals

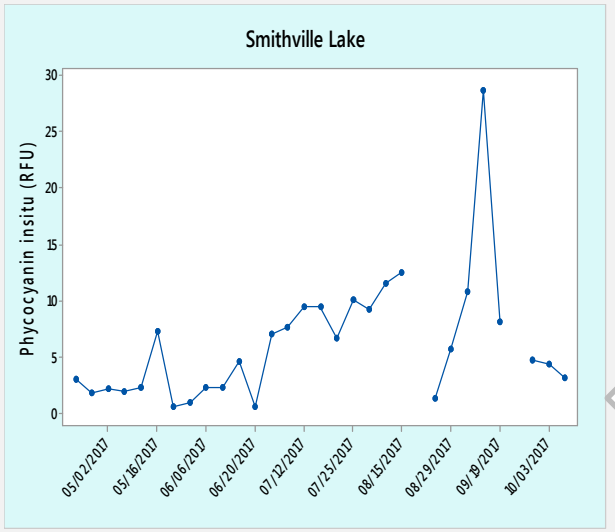


CYAN Project
USGS, NOAA, EPA, NASA

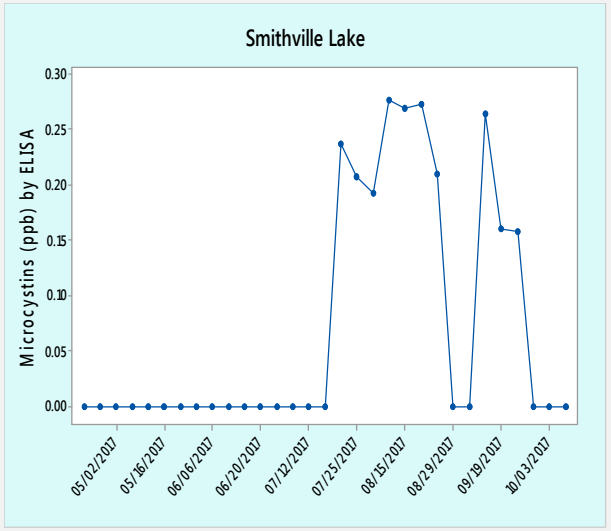




Microcystin in Smithville Lake

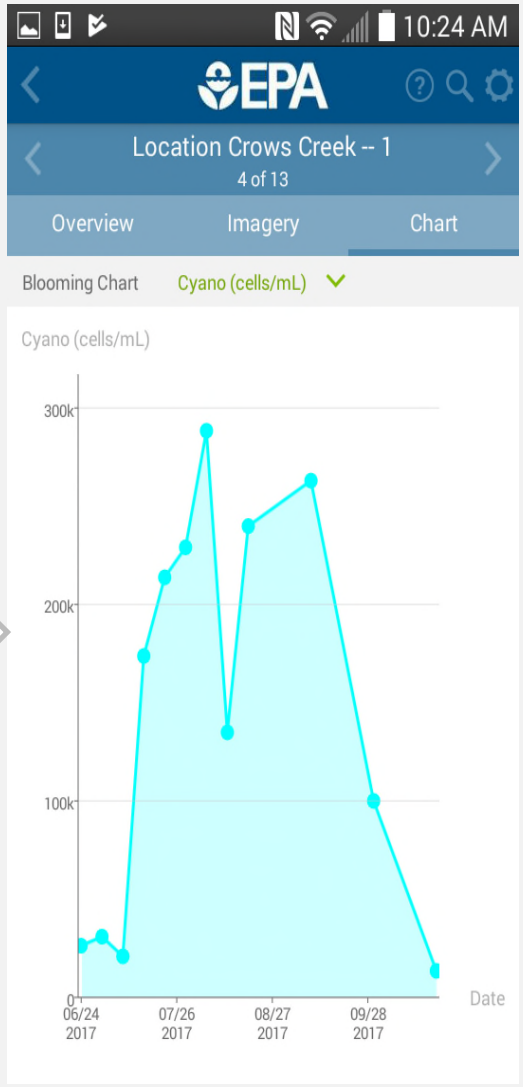


Phycocyanin begins to increase in early July



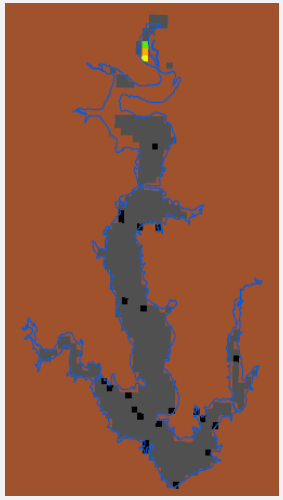
Detect Microcystin mid-July through September

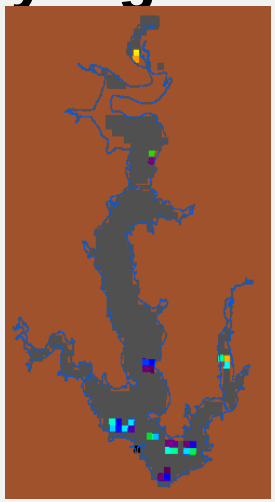
CYAN smartphone app shows a signal

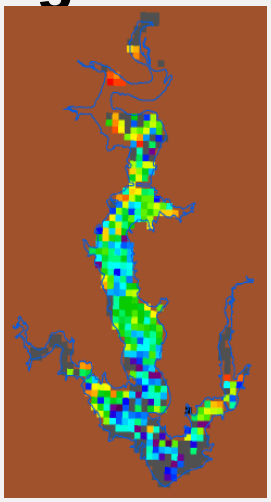


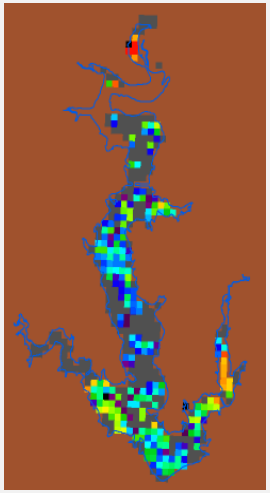
Note: toxin concentration never near harmful levels

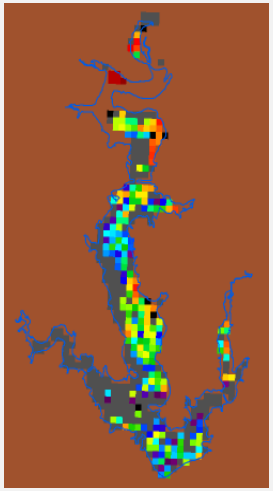
Lake Perry – growing season 2017

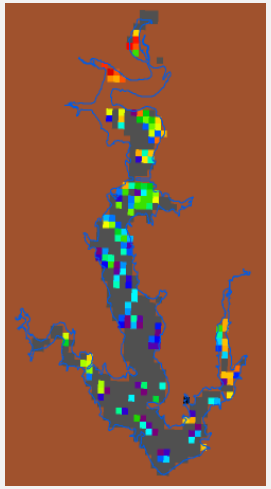

7/2-8

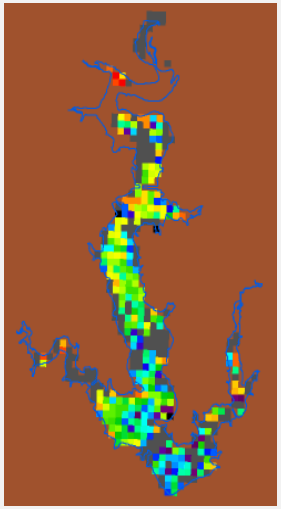

7/9-15

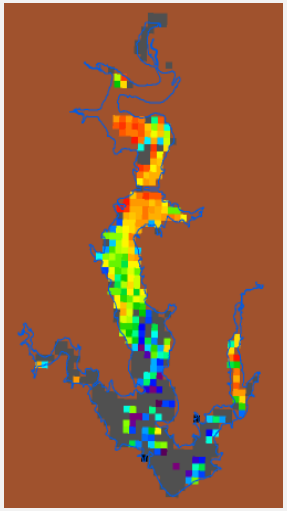

7/16-22

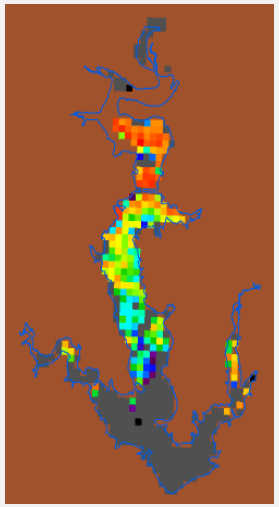

7/23-29

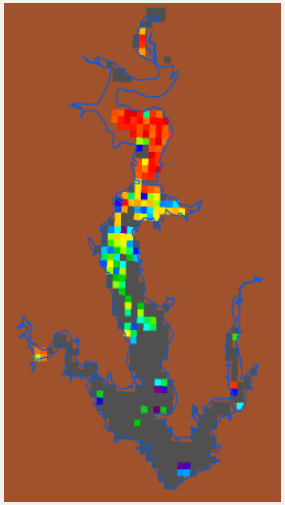

7/30-8/5

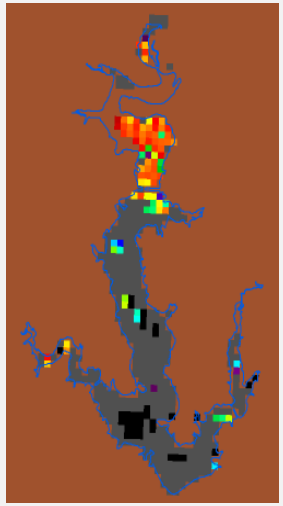

8/6-12


8/13-19


8/20-26

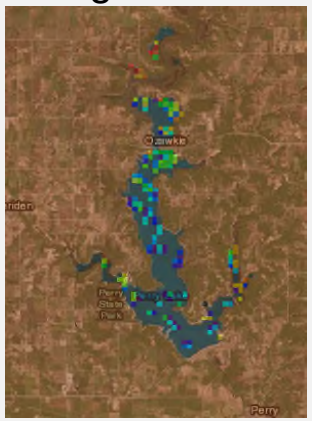

8/27-9/2


9/3-9

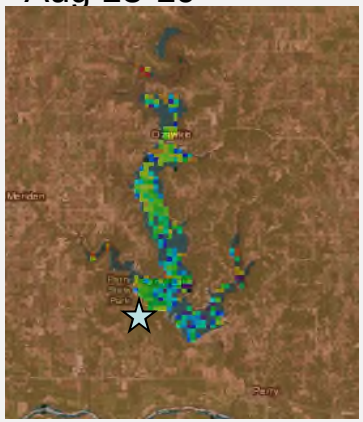

9/10-16

EPA United States Environmental Protection Agency

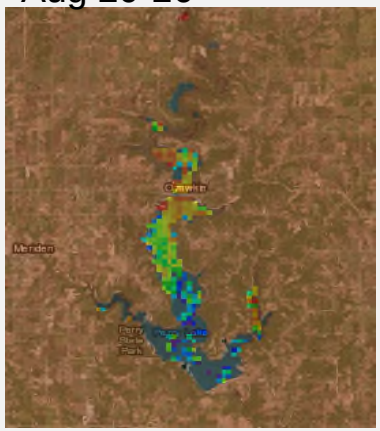
Aug 6-12

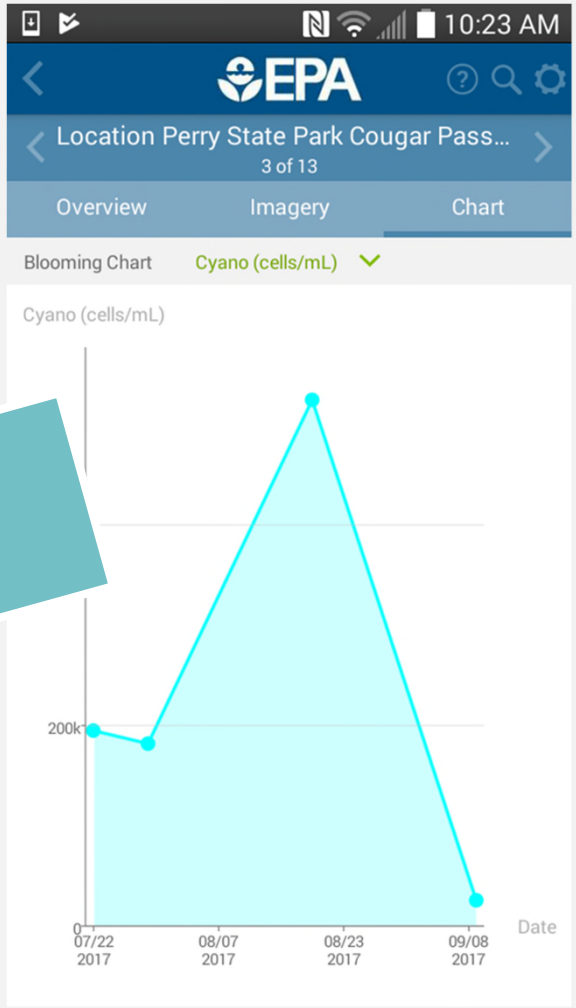


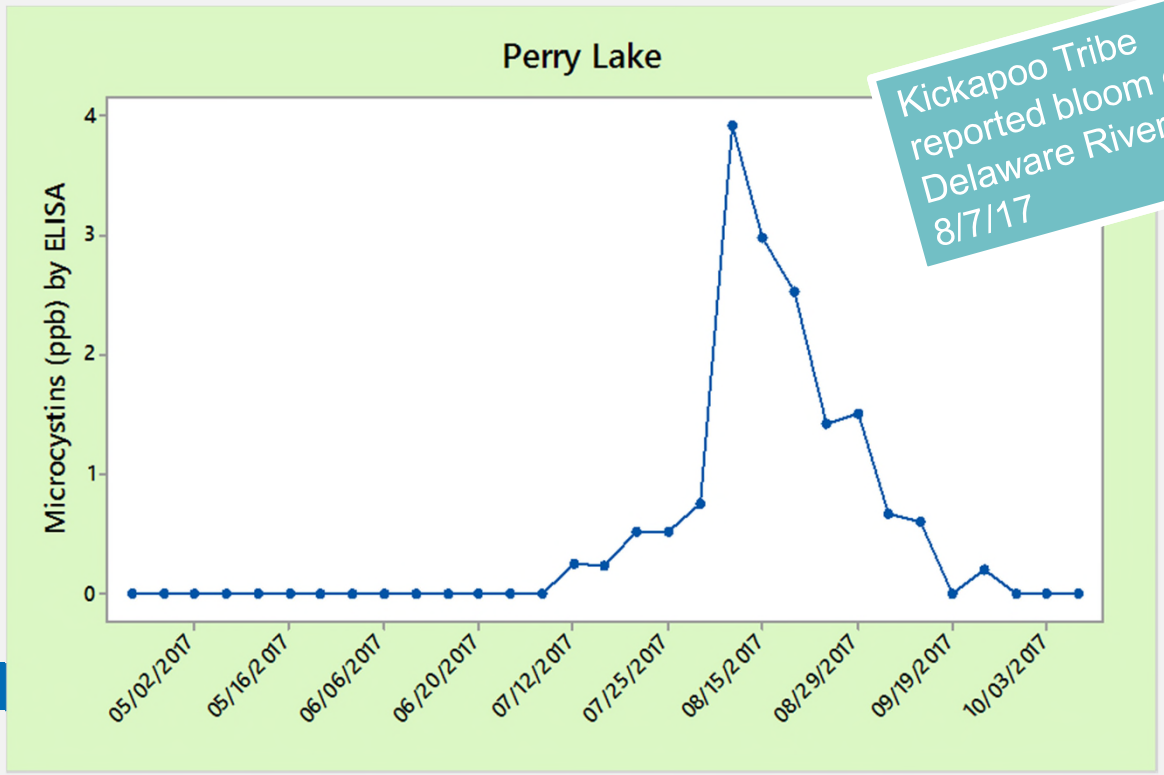
Aug 13-19



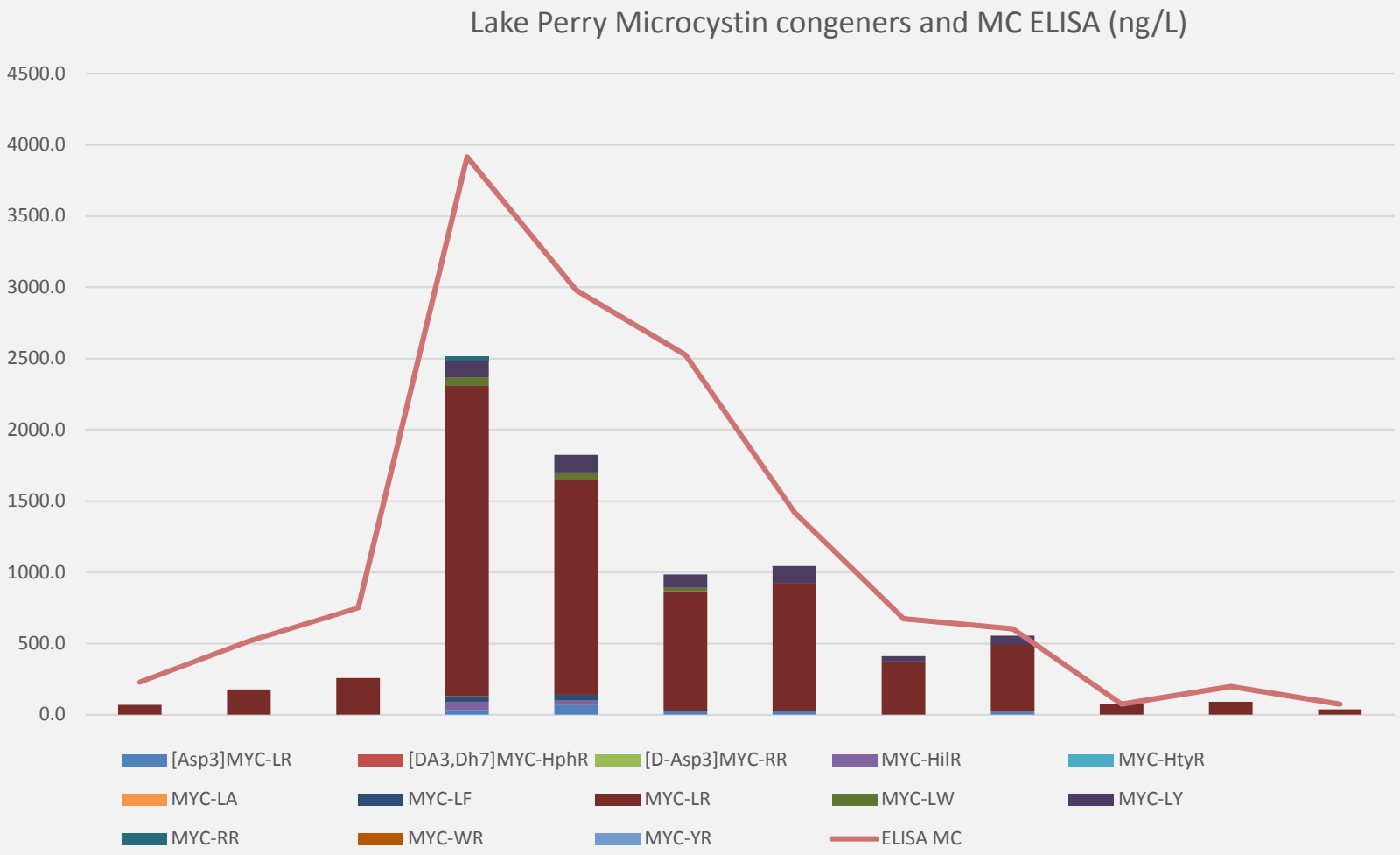
Aug 20-26

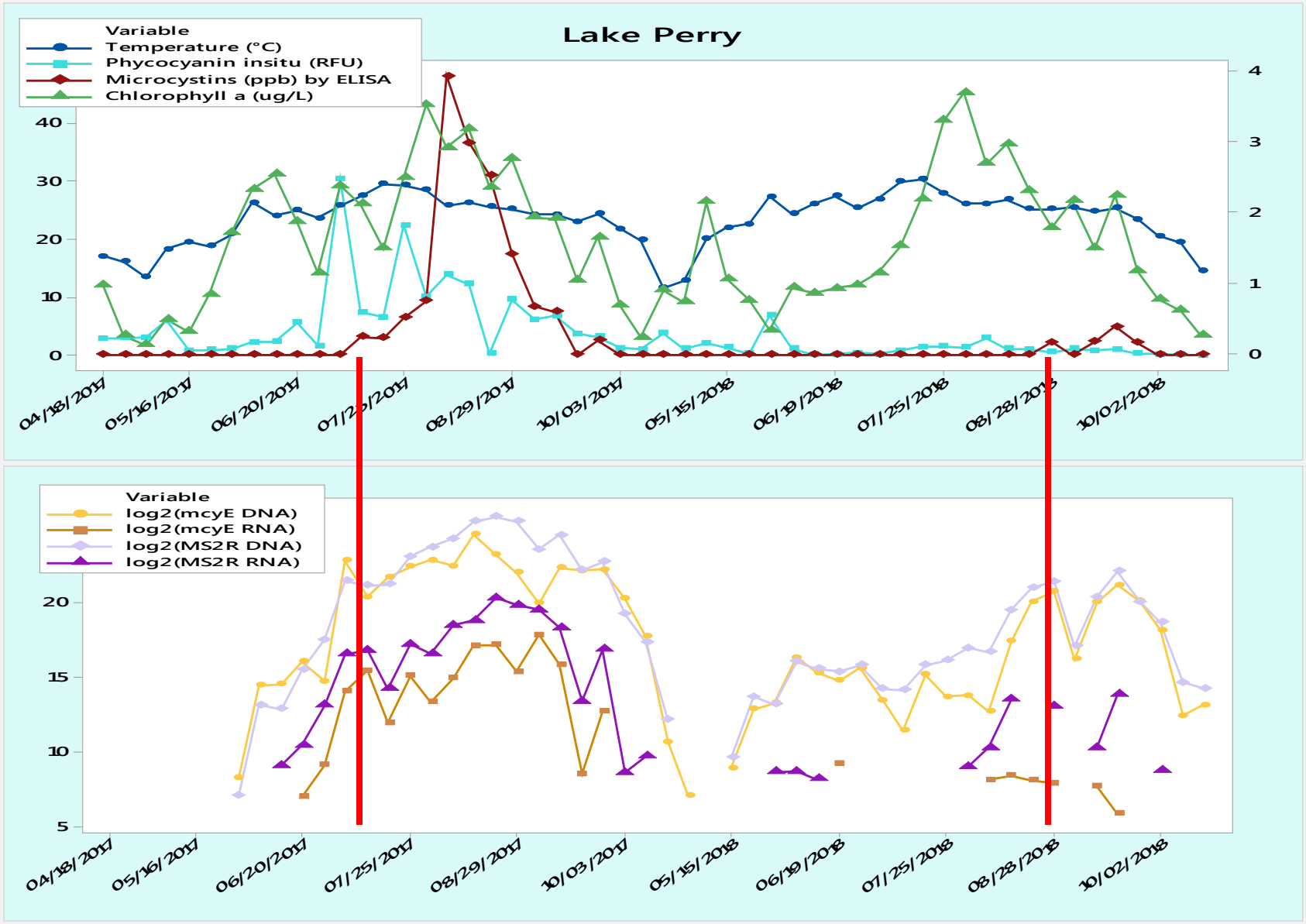






Kickapoo Tribe reported bloom on Delaware River on 8/7/17


Lake Perry Microcystin congeners and MC ELISA (ng/L)
4500.0
4000.0
3500.0
3000.0
2500.0
2000.0
1500.0
1000.0
500.0
0.0
[Asp3]MYC-LR
[DA3,Dh7]MYC-HphR
[D-Asp3]MYC-RR
MYC-HilR
MYC-HtyR
MYC-LA
MYC-LF
MYC-LR
MYC-LW
MYC-LY
MYC-RR
MYC-WR
MYC-YR
ELISA MC


Lake Perry
Variable
Temperature (°C)
Phycocyanin insitu (RFU)
Microcystins (ppb) by ELISA
Chlorophyll a (ug/L)
40
30
20
10
0
4
3
2
1
0
04/18/2017
05/16/2017
06/20/2017
07/25/2017
08/29/2017
10/03/2017
05/15/2018
06/19/2018
07/25/2018
08/28/2018
10/02/2018
Variable
log2(mcyE DNA)
log2(mcyE RNA)
log2(MS2R DNA)
log2(MS2R RNA)
20
15
10
5
04/18/2017
05/16/2017
06/20/2017
07/25/2017
08/29/2017
10/03/2017
05/15/2018
06/19/2018
07/25/2018
08/28/2018
10/02/2018

EPA United States Environmental Protection Agency

Next:

- Develop the RNA/DNA qPCR methods regionally
- Correlate chemistry, physical, biological and molecular data
- 2019/2020 RARE for Milford Lake

Special thanks to our sampling team!!!